PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
G01N 33/541, 33/53, 29/02
B01J 19/10

A2

(11) International Publication Number: WO 93/19367

(43) International Publication Date: 30 September 1993 (30.09.93)

(21) International Application Number: PCT/GB93/00504

(22) International Filing Date: 10 March 1993 (10.03.93)

(30) Priority data:
9205128.3 10 March 1992 (10.03.92) GB

(71) Applicant *(for all designated States except US)*: UNIVERSITY COLLEGE CARDIFF CONSULTANTS LIMITED [GB/GB]; Park Place, Cardiff CF1 1XL (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : COAKLEY, William, Terence [GB/GB]; 30 Windmere Avenue, Cardiff CF2 5PR (GB). GRUNDY, Martin, Alan [GB/GB]; 15 Stranraer Road, Pennar, Pembroke Dock, Dyfed SA72 6RY (GB). BOLEK, Werner [AT/AT]; Wiedner Haupstrasse 8-10/134, A-1040 Wien (AT).

(74) Agent: GIBSON, Stewart, Harry; Urquhart-Dykes & Lord, Business Technology Centre, Senghennydd Road, Cardiff CF2 4AY (GB).

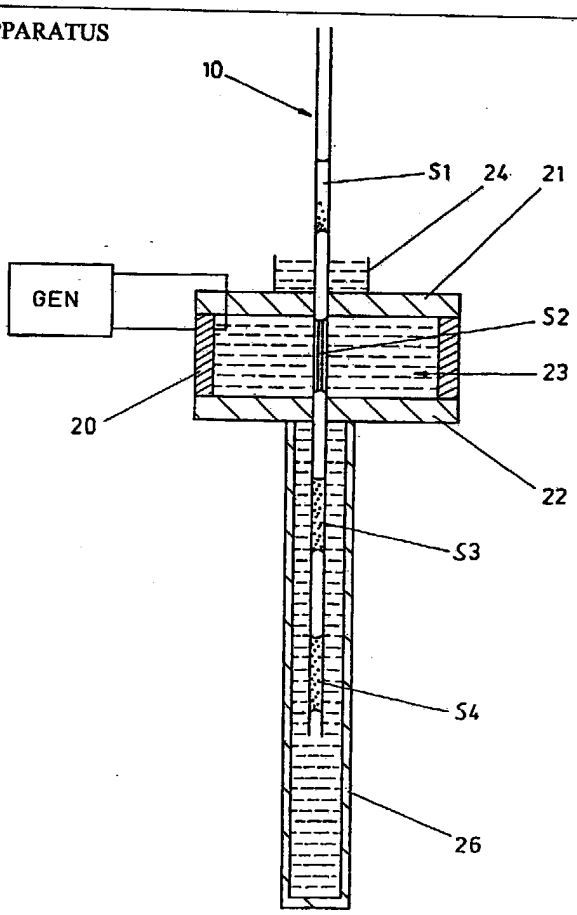
(81) Designated States: AU, CA, FI, HU, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: PARTICLE AGGREGATION METHOD AND APPARATUS

(57) Abstract

A sample (e.g. 52) of liquid with suspended particles is contained in a tube (10) and subjected to a standing wave ultrasound field transverse to the tube (10), the standing wave exhibiting a progressive change in pressure amplitude transverse to the tube, so that particles in suspension are displaced transversely of the tube to one or more predetermined regions: exposure of the sample to the standing wave is then terminated and the particles are allowed to settle, and inspected to determine whether they remain aggregated or whether they dissociate. The ultrasound field is produced by a transducer (20) of tubular form encircling the tube (10). The invention may be used for the agglutination of particles or cells via cross-bridging molecules in immuno-agglutination assays.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SK	Slovak Republic
CI	Côte d'Ivoire	KZ	Kazakhstan	SN	Senegal
CM	Cameroon	LI	Liechtenstein	SU	Soviet Union
CS	Czechoslovakia	LK	Sri Lanka	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	MC	Monaco	UA	Ukraine
DK	Denmark	MG	Madagascar	US	United States of America
ES	Spain	ML	Mali	VN	Viet Nam
FI	Finland	MN	Mongolia		

PARTICLE AGGREGATION METHOD AND APPARATUS

This invention relates to a method and apparatus for the aggregation of particles, more particularly for the agglutination of particles via cross-bridging molecules as occurs for example in immuno-agglutination assays, where cross-bridging is via antibody molecules.

GB 2 233 089 discloses a method and apparatus for monitoring the aggregation of particles suspended in a liquid, in which a sample of the liquid is subjected to ultrasound to promote aggregation and possible agglutination of particles, and then the particles are allowed to settle onto a meniscus of the sample. A visual inspection of the particles once settled on the meniscus enables a determination of whether an agglutination reaction has occurred: agglutinated cells lie in clumps distributed over the meniscus, whereas clumps of non-agglutinated particles dissociate and form a ring around the edge of the meniscus.

The method and apparatus disclosed in GB 2 233 089 is particularly applicable to the detection of Hepatitis B virus, a reaction mixture being formed of a serum sample plus a suspension of appropriate erythrocytes coated with antibody to Hepatitis B surface antigen. Viral agglutination of the erythrocytes gives a positive test reaction.

In GB 2 233 089, ultrasound is coupled via a reservoir into the bottom of a vertical capillary tube containing the sample. The arrangement is not suited to multiple-sample testing because the reservoir, in which the capillary tube stands, must be washed out and refilled for each test; also continuous movement of successive samples through the capillary tube is impractical because the ultrasonic transducer is positioned across one end of the capillary tube.

We have now devised an improved method and apparatus for rapid aggregation of particles.

In accordance with this invention, there is provided a method for the aggregation of particles suspended in a liquid, comprising containing a sample of the liquid in a tube, generating a standing wave ultrasound field transverse to the tube, the standing wave exhibiting a progressive change in pressure amplitude transverse to the tube, so that particles

in suspension are displaced transversely of the tube to one or more predetermined regions, and then terminating the exposure of the sample to the standing wave and allowing the particles to settle.

Once the particles have settled, they are inspected to determine whether they have remained aggregated or whether they have dissociated. Alternatively, or in addition, the particles can be inspected whilst they are in the process of settling. In any event, the inspection can be carried out by the eye, or by directing a beam of light at the particles and using a sensor to respond to the reflected or transmitted beam.

Preferably the standing wave exhibits a progressive change in pressure amplitude from a first region within the tube to said predetermined region or regions, which are spaced inwards from the inner surface of the tube but preferably are closer to the inner surface than said first region. For example the standing wave may exhibit a pressure amplitude maximum at or adjacent a longitudinal axis of the tube and a pressure amplitude minimum at a said predetermined region spaced inwards from the inner surface of the tube.

Preferably the ultrasound standing wave field extends radially outwards from a longitudinal axis of the tube in different angular directions, and preferably in all angular directions through 360°. Preferably the ultrasound standing wave field is generated by a transducer of tubular form which encircles the tube containing the liquid sample.

Preferably the sample of liquid has a meniscus at its lower boundary within the tube, and the particles are allowed to settle onto this meniscus.

The arrangement enables the sample tube to contain several liquid samples, separated by bubbles of air or other fluid, so that the sample tube can be displaced longitudinally, taking the successive samples through the ultrasound field and then into a position where the particles can settle. Instead, successive samples separated by bubbles of air or other fluid can be caused to flow through the sample tube.

Also in accordance with this invention, there is provided an apparatus for the aggregation of particles, comprising a tube for containing a liquid sample, and an

ultrasonic transducer arranged to generate a standing wave ultrasound field transverse to the tube, the standing wave exhibiting a progressive change in pressure amplitude transverse to the tube, so that, in use of the apparatus, particles in suspension are displaced transversely of the tube to one or more predetermined regions.

The tube may be disposed generally vertically so that the particles will settle onto the lower meniscus of the liquid sample. Instead, the tube may be inclined at an angle to the vertical, so that the particles settle onto a side wall of the tube and/or into a corner between the side wall and the meniscus.

Embodiments of this invention will now be described by way of examples only and with reference to the accompanying drawings, in which:

Figure 1 is a schematic vertical section through an apparatus for performing an immuno-agglutination assay in accordance with the invention; and

Figure 2 is a similar section through a modified apparatus in accordance with the invention.

Referring to Figure 1 of the drawing, there is shown a vertically-disposed glass capillary tube 10 containing a number of samples S1 to S4 separated by air spaces. A piezoelectric ultrasonic transducer 20, of circular-section tubular shape, encircles the capillary tube coaxially. Two discs 21,22 of e.g. perspex are sealed across the top and bottom of the transducer to form a housing 23 which is filled with water. The capillary tube 10 passes in a close-fit manner through holes in the centres of the discs 21,22. An elongate tube 26 is sealed to the underside of the lower disc 22 and is also filled with water. A reservoir 24 of water is provided on the top of the upper disc 21 and serves to prevent air being introduced into the housing 23 during displacement of the capillary tube 10.

The transducer 20 is provided with electrodes over its inner and outer surfaces and an a.c. generator GEN is connected across these electrodes to drive the transducer in its thickness mode. Accordingly, a standing wave ultrasound field is generated, extending radially outwards in all angular

directions from the longitudinal axis of the capillary tube 10. The standing wave exhibits a primary pressure amplitude maximum substantially on the axis of the capillary tube, and successive maxima, of lower amplitude, at half-wavelength intervals radially outwards from the tube axis. The frequency of the a.c. excitation signal is selected, having regard to the internal diameter of the capillary tube, so that the first pressure amplitude minimum lies at a position spaced radially inwards from the inner surface of the capillary tube. Thus in use, particles in the liquid sample, which is disposed in the ultrasound field, are displaced to an annular region at the position of the first pressure amplitude minimum.

The ultrasound field thus causes aggregation of the particles at the annular region of the first pressure amplitude minimum and furthermore promotes any possible agglutination.

In use, a number of liquid samples are contained within the capillary tube separated by the spaces filled with air or other fluid, each sample including, for example when testing for Hepatitis B virus in blood, serum and a suspension of appropriate erythrocytes coated with antibody to Hepatitis B surface antigen. Agglutination is promoted by the ultrasound field in the case of a positive sample, when the serum contains Hepatitis B virus. The capillary tube 10 is inserted until the top sample lies within the transducer housing 23, and the a.c. drive signal is connected to the transducer for e.g. 15 seconds. After this, the capillary tube is slid upwards until the second-from-top sample lies within the transducer housing 23, and the transducer is switched on for another e.g. 15 second period. The particles in the top sample are now able to settle under gravity onto the lower meniscus of this sample, and the meniscus can be observed. As mentioned above, if agglutination occurs, the meniscus exhibits a granular appearance with groups or clumps of particles spread over the meniscus surface, otherwise the clumps of particles or cells disperse or disaggregate as they settle and form a smooth or uniform ring around the edge of the meniscus. Thus, visual inspection shows whether the test has proved positive or negative.

It will be appreciated that the method which has been

described enables several samples to be contained within a single capillary tube and tested one-after-another. The step-wise displacement of the capillary tube may be effected manually or automatically. In a modification, it is possible to cause successive samples, separated by gaps of air or other fluid, to flow through a fixed capillary tube disposed on the axis of the ultrasound transducer.

Whilst the use of a capillary tube in a generally vertical disposition has been described, the capillary tube may instead be inclined at an angle to the vertical, as shown in Figure 2. The particles will then settle under gravity onto the side wall of the tube and/or into the corner between the tube wall and the meniscus, as shown at P. The particles can then be inspected at the location(s) where they have settled: as mentioned previously, inspection may be carried out by directing a beam of light (e.g. from a laser 30) at the particles and using a sensor e.g. 32 to pick up the transmitted beam.

Also, whilst the method and apparatus have been described with reference to the agglutination of particles or cells via cross-bridging molecules, the method and apparatus are applicable to other uses where it is desired to test the extent to which particles link or bond together when aggregated, e.g. in the case of flocculation. However, it will be appreciated that in all of the cases which have been described, the nature of the particles is such that they do not bond or adhere to the inner surface of the tube, should they come into contact with that surface, but instead they slide down that surface when allowed to settle under gravity upon removing the ultrasonic field.

Further, whilst the method and apparatus have been described as using an ultrasonic transducer of tubular form encircling the sample tube, transducers of other forms may be envisage, e.g. a planar transducer, to provide a standing wave ultrasound field transverse to the tube.

Claims

1) A method for the aggregation of particles suspended in a liquid, comprising containing a sample of the liquid in a tube, generating a standing wave ultrasound field transverse to the tube, the standing wave exhibiting a progressive change in pressure amplitude transverse to the tube, so that particles in suspension are displaced transversely of the tube to one or more predetermined regions, and then terminating the exposure of the sample to the standing wave and allowing the particles to settle.

2) A method as claimed in claim 1, further comprising the step of inspecting the particles whilst in the process of settling and/or once they have settled.

3) A method as claimed in claim 1 or 2, in which the sample of liquid has a meniscus at its lower boundary within the tube, and the particles are allowed to settle onto said meniscus.

4) A method as claimed in claim 1 or 2, in which said tube is included to the vertical and the particles are allowed to settle onto the inner side surface of the tube and/or into a corner between the tube side surface and a meniscus of the liquid at its lower boundary.

5) A method as claimed in any preceding claim, in which said standing wave exhibits a progressive change in pressure amplitude from a first region within the tube to said predetermined region or regions, said predetermined region or regions being spaced inwards from the inner surface of the tube but closer than said first region to said inner surface.

6) A method as claimed in any preceding claim, in which the tube contains a plurality of liquid samples separated by bubbles of air or other fluid and the successive samples are stepped, longitudinally of the tube, through the ultrasound field.

7) An apparatus for the aggregation of particles suspended in a liquid, comprising a tube for containing a sample of said liquid, and an ultrasonic transducer arranged to generate a standing wave ultrasound field transverse to the tube, the standing wave exhibiting a progressive change in pressure amplitude transverse to the tube, so that, in use of the apparatus, particles in suspension are displaced transversely of the tube to one or more predetermined regions.

8) An apparatus as claimed in claim 7, in which the tube is disposed vertically so that the particles will, in use of the apparatus, settle onto a meniscus at a lower boundary of the liquid sample.

9) An apparatus as claimed in claim 7, in which the tube is inclined to the vertical, so that the particles will, in use of the apparatus, settle onto an inner side surface of the tube and/or into a corner between said inner side surface and a meniscus at a lower boundary of the liquid sample.

10) An apparatus as claimed in any one of claims 7 to 9, in which said ultrasonic transducer comprises a transducer of tubular form encircling said tube.

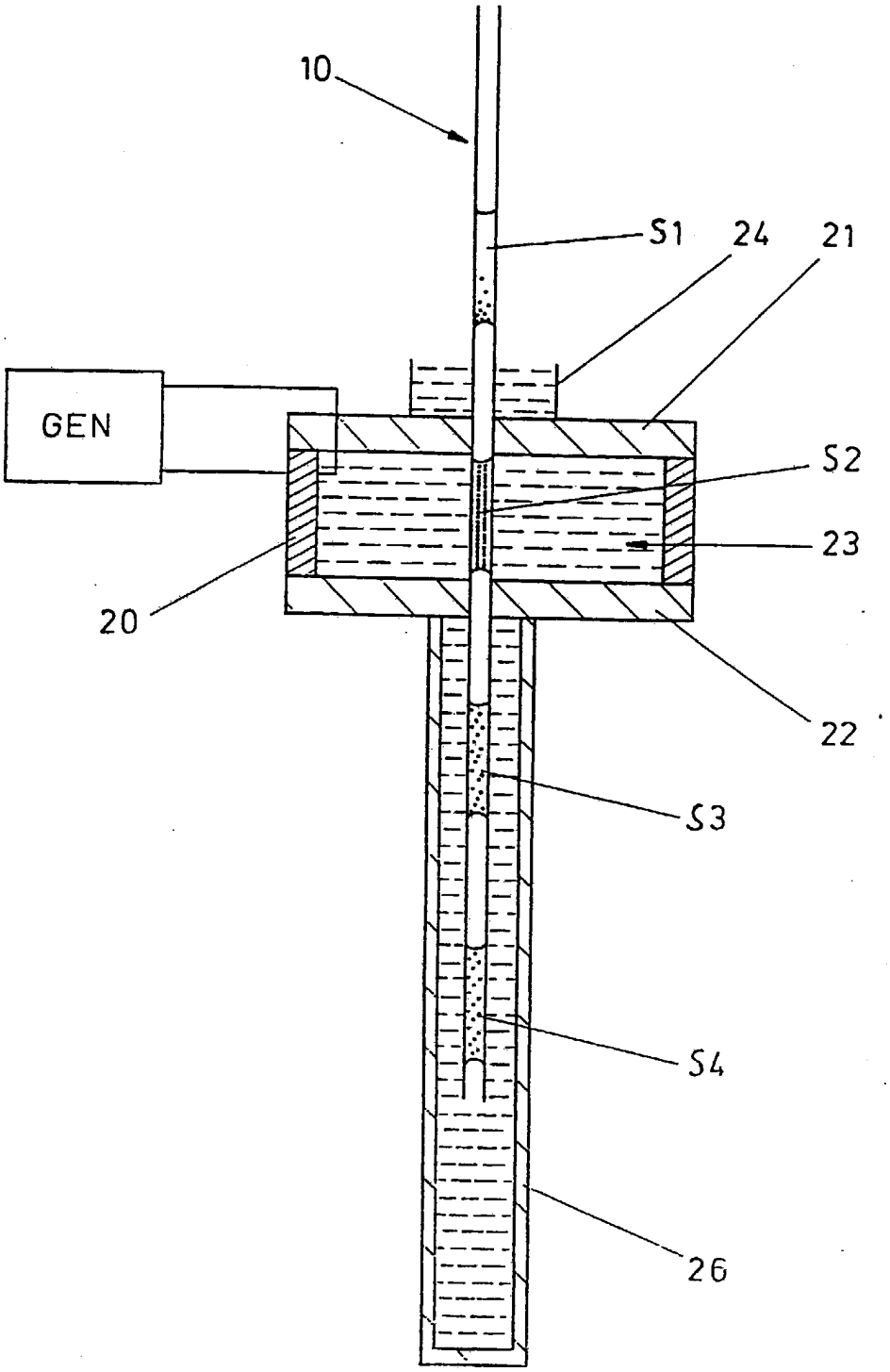


FIG. 1

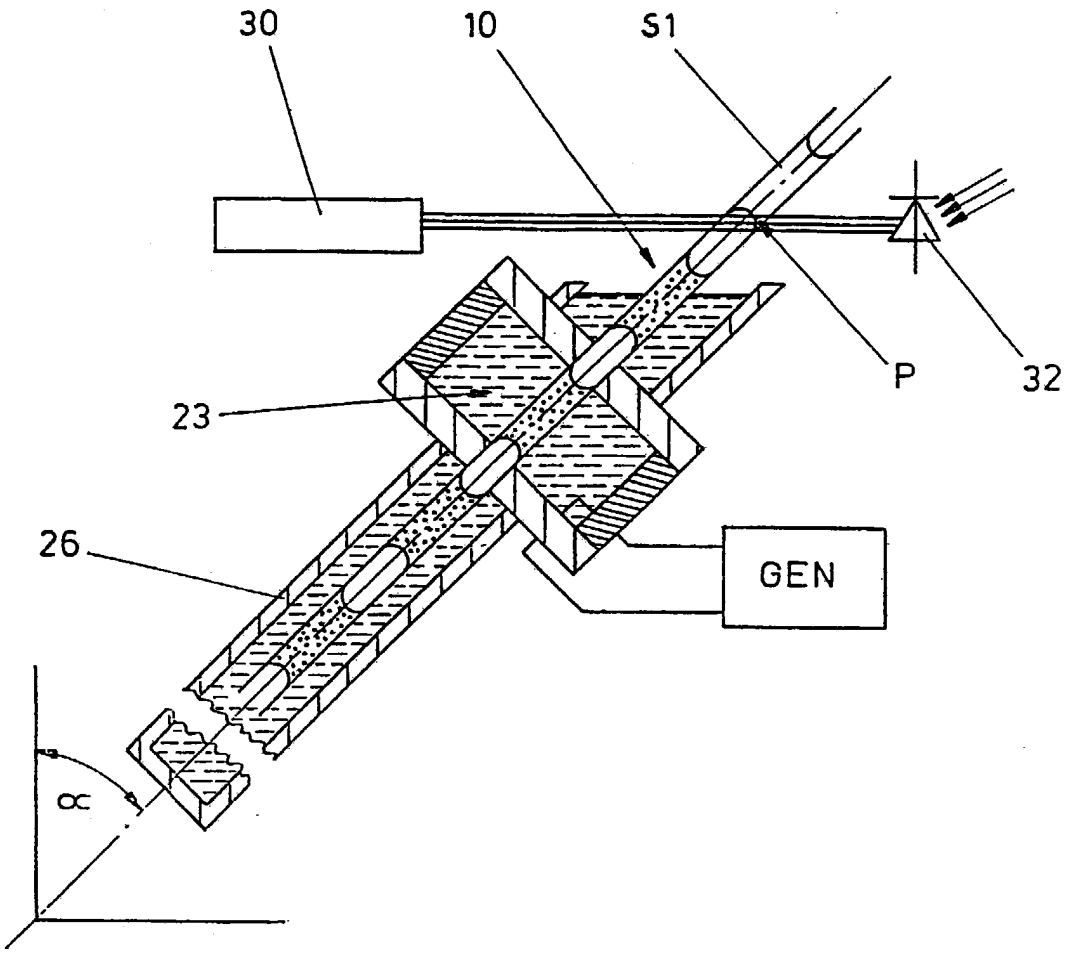


FIG. 2